Effect of Environmental Stress Factors and Recycling on the Lipid Composition of Brewer's Yeast Mitochondria

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Abstract

The aim of this study was to investigate alterations in the content and composition of mitochondrial lipids of brewer's yeast, which occur during brewing and repetitive recycling. The bottom-fermenting brewer's yeast of *Saccharomyces cerevisiae* species obtained from industrial beer production was used throughout the study. The first three generations of recycled yeast were analysed. Differences between the generations were more pronounced in the phospholipid and neutral lipid composition than in fatty acid composition. Squalene was present in all generations in high concentrations. The results give insight into the response of yeast cells to stress factors and recycling.

Keywords

Brewer's yeast, recycling, mitochondria, plasma membrane, lipids, squalene

1 Introduction

Water, malt, yeast, and hops are the basic raw materials for the production of beer. Yeast is responsible for the production of alcohol and carbon dioxide, but also for the production of fermentation by-products (higher alcohols, acids and esters), which are responsible for the bouquet of beer.[1]

Most organisms are exposed to constant changes in their environment, and therefore their survival depends on the capacity to adapt to new conditions. This also applies to industrial yeasts, but in a much more pronounced way, since their cells are exposed simultaneously to different stressors during production processes. Among them, exposure to ethanol and oxygen-limited availability are of special importance for the bottom-fermenting brewer's yeast.[2,3] Modern brewing technology is based on wort fermentation with selected, aerobically propagated, pure yeast culture. During aerobic growth, some yeasts (like those from *Saccharomyces* genus) in the second growth phase (called oxidative), spend ethanol produced from sugars in the first phase (called fermentative).[4] Yeast culture used in one fermentation cycle, is usually reused in successive fermentation cycles. The number of cycles is theoretically unlimited, but in practice, it includes 8–10 fermentation cycles (often reduced to 3–5). The performance of brewer's yeast declines with repeated use and the yeast is said to become "weak" and physiologically inactive. Recycled yeast is pitched in wort with limited oxygen concentration. In the case of the bottom-fermenting brewer's yeast, characterized by high density, the aerobically propagated pure yeast culture undergoes anaerobic conditions during fermentations because the yeast biomass precipitates on the bottom of fermenter. Failure to provide sufficient molecular oxygen to the fermentation medium restricts yeast growth and viability, since the cells cannot produce unsaturated fatty acids (FAs) and sterols for membrane biosynthesis.[5–7] Yet, brewing wort contains small portions of unsaturated FAs, which the yeast cells can use for membrane reconstitution.[8]

Various physiological adaptations related to stress protection occur in yeasts, ranging from the alteration in membrane fluidity to the synthesis of detoxification enzymes.[5,6,9] Membrane lipids play an essential role in the biophysical and biochemical characteristics of cell membranes; they determine permeability and fluidity, enable proper functioning of membrane-attached proteins, and participate in the cell signalling. The ability of yeast cells to alter sterol to phospholipid (PL) ratio, PL composition and location, as well as the degree of unsaturation of membrane lipids is an important factor in adaptation to environmental conditions.[7,10,11]

The mitochondria are engaged in many cellular processes, one of the most important being oxidative degradation of nutrients for the production of energy. Since the cell growth and normal functioning depend on the generation of metabolic energy, well-developed mitochondria are of special importance for the cells. They are dynamic organelles whose functional integrity requires a coordinated supply of proteins and lipids adjusted to meet physiological and functional demands.[12] Their lipid composition is of great importance since they contain a double membrane system, with a pronounced curvature of the inner membrane shaped by lipids.

Phosphatidylcholine (PtdCho), phosphatidylethanolamine (PtdEtn) and phosphatidylinositol (PtdIns) are the main phospholipids in *S. cerevisiae* cells, including the mitochondria. Cardiolipin (CL), a typical mitochondrial phospholipid, is the key determinant in the maintenance of

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mtDNA stability and segregation.[13] Ergosterol, the main and essential neutral lipid present in all membranous systems of yeasts, modulates membrane fluidity and permeability. It is highly enriched in the plasma membrane (PM), while triacylglycerols (TAGs) and steryl esters (SEs), representing reserve lipid molecules, are mainly located in the lipid particles. Squalene, another neutral lipid, is a triterpenic hydrocarbon formed in the first part of sterol biosynthetic pathway – the so-called mevalonate pathway – and further converted to squalene epoxide by squalene epoxidase, Erg1p.[14] This step requires oxygen, making sterol synthesis strictly aerobic. According to our findings for another strain of brewer's yeast (obtained from another brewing company), in anaerobic conditions, the bottom-fermenting brewer's yeast incorporates squalene in its membranes and thus partially replaces ergosterol as its adaptation to the governing conditions during beer production.[15] Specific characteristics of the mitochondria, compared to other subcellular fractions, are also low PL to protein and sterol to protein ratios.[16] Considering the FA composition of *S. cerevisiae*, the major FAs are palmitic (16 : 0), palmitoleic (16 : 1Δ^9), stearic (18 : 0) and oleic (18 : 1Δ^9) acid. Since the only FA desaturase in *Saccharomyces* is the Δ-9 desaturase (Ole1), yeasts do not contain polyunsaturated FAs unless they are added exogenously.

A number of studies has documented the alterations of cell lipids in the yeasts exposed to ethanol or lack of oxygen in laboratory conditions,[7,17,18] which are usually far from the conditions in industrial processes. We have published data on the lipid composition of the bottom-fermenting brewer's yeast obtained in the industrial process of beer production in another brewing company, its cells and organelles[19,20] and data on the alterations of several lipid parameters caused by recycling of the yeast analysed in this study.[21–23] In the present study, we analysed the alterations in the lipid composition of the mitochondria, which occur during propagation, brewing and repetitive recycling of the yeast biomass. The results provide a better insight into the capability of brewer's yeast to adapt to stress conditions because each of the three phases the brewer's yeast passes through during beer production (propagation, fermentation and storage) and also the transition from one phase to another, include a number of stresses. The content and composition of phospholipids, the neutral lipids and fatty acid composition in total mitochondrial lipids are presented. Because of an unusual neutral lipid composition of the mitochondria, we analysed the plasma membranes, and checked whether other membranous systems in the brewer's yeast shared a similar pattern.

2 Experimental

2.1 Yeast strain

The bottom-fermenting brewer's yeast or lager brewing strain of the species *Saccharomyces cerevisiae*, used throughout the study, was provided from a local brewery. It is deposited at the Collection of Microorganisms of the University of Weinstephan, Munich, Germany. The starter yeast culture and cultures of the first three recycled yeast generations were analysed. Briefly, they were obtained as follows: the laboratory-grown culture was transferred into the yeast propagator containing fresh malt wort continuously aerated. Aerobically propagated starter yeast culture (designated the zero-yeast generation) was pitched into hopped brewery wort in the fermenter. The fermentation process was carried out anaerobically. The yeast harvested after the fermentation cycle was pitched in the pre-aerated wort, thus providing oxygen required for several biosynthetic reactions during the first phases of fermentation, and then reused in the subsequent cycle. The process is described in detail in *Čanadi Jurešić and Blagović* (2011).[23]

Prior to any experiment, the biomass was washed three times with 0.1 % $NaHCO_3$ in order to eliminate the bitter components of hops, and centrifuged at 3000 *g* for 5 min.

2.2 Isolation of mitochondria

Crude mitochondria were isolated by differential centrifugation after enzymatic disruption of the cell wall using zymoliase.[24]

2.3 Plasma membrane preparation

The plasma membranes were obtained by the method for the isolation of highly purified plasma membranes.[25] For technical reasons, the first part of the method was modified, so that instead of mechanical disruption of the cell wall with glass beads, the enzymatic method with zymoliase was applied, described in detail in our previous paper.[23]

2.4 Protein determination

Protein content of the plasma membrane was determined by the method of *Lowry*[26] using bovine serum albumin as the standard.

2.5. Lipid extraction

Total lipids from the mitochondria and plasma membrane were extracted by Folch method.[27]

Chromatographic methods used for the lipid analysis are the standard ones for the determination of basic lipid composition and accepted by *Saccharomyces cerevisiae* – EUROFAN (European Function Analysis Network) 2 group.[28]

2.6 Phospholipid analysis

Total phospholipids (TPLs) in the lipid extract were determined spectrophotometrically as inorganic phosphorus. Samples of total lipid extracts were digested with concentrated nitric and sulfuric acid by gentle heating in a microwave oven for 20 min. After cooling, the solution was neutralized with solution of sodium hydroxide. The intensity of blue colour, developed by ascorbic acid, solution of ammonium heptamolybdate tetrahydrate and antimony potassium tartrate hemihydrate in sulfuric acid, was measured at 880 nm. The concentration of TPLs was calculated by multiplying mass concentration of phosphorus by 25 and expressed *per* mass of proteins. PL classes were separated by two-dimensional thin-layer chromatography on silica gel 60 plates, 20 × 20 cm, 0.2 mm. Chloroform/methanol/ammonia (in a volume ratio 13 : 7 : 1) was used as the first, and chloroform/acetone/methanol/acetic acid/

water (in a volume ratio 10 : 4 : 2 : 2 : 1) as the second solvent system. PLs were visualised by iodine staining, scraped off the plate, and quantified the same way as TPLs, using factor 27.1 for PtdIns, and 24.2 for other PLs, respectively.

2.7 Analysis of neutral lipids

Neutral lipids were separated by two-step TLC on silica gel 60 plates (200 × 100 × 0.2 mm). Lipid extracts and standards were applied by a sample applicator (Linomat V, Camag, Switzerland). The plates were developed by using light petroleum/ diethyl ether/ CH_3COOH (volume ratio 20 : 20 : 0.8) up to 1/3 of a plate as the first, and light petroleum/ diethyl ether (volume ratio 39.2 : 0.8) up to 2/3 of plate as the second solvent system. For the determination of components, the bands were visualised by post chromatographic derivatization. Plates were dipped for 4 s into developing reagent (0.63 g $MnCl_2 \cdot 4H_2O$, 60 ml H_2O, 60 ml CH_3OH and 4 ml H_2SO_4, conc.), dried, and heated for 30 min at 100 °C. Quantification was carried out by densitometric scanning (GS-710 Calibrated Imaging Densitometer, Bio-Rad).

2.8 Fatty acid analysis

Fatty acid (FA) composition was determined by GC analysis of the corresponding methyl esters obtained by acid methanolysis of lipid extracts with BF_3/methanol. GC analyses of FA methyl esters were carried out using an Auto System XL from Perkin-Elmer with flame-ionization detector (FID). An SP-2330 capillary column (Supelco, USA), 30 m × 0.32 mm × 0.2 μm and helium as carrier gas with split injection (100 : 1) were used. Hydrogen was obtained with a Claind hydrogen generator. The analyses were carried out in programmed temperature mode from 140 to 220 °C at 5 °C min^{-1} and then isothermally for 25 min. The injector temperature was 300 °C and the detector 350 °C. For data acquisition, Chromatography Software from Perkin-Elmer Nelson (Turbochrom 4) was used. The results were expressed as mass fractions of individual FA in total FAs. The degree of unsaturation is expressed as unsaturation index (UI) which is calculated as follows:

$$UI = w(\text{monoenoic fatty acids}) + 2\,w(\text{dienoic fatty acids}) + 3\,w(\text{trienoic fatty acids}).^{15}$$

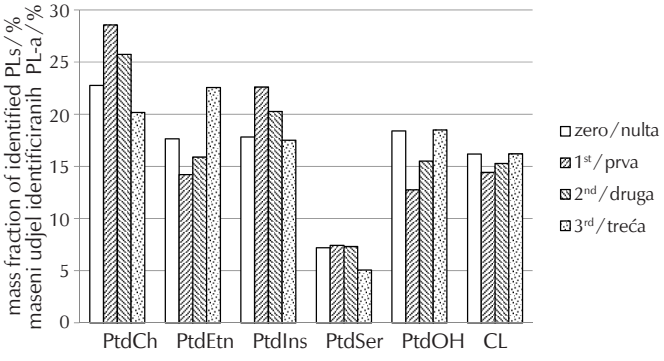
3. Results and discussion

Saccharomyces cerevisiae is used in beer production in batch fermentation, where it is recycled several times. During transition from propagation – performed in aerobic conditions – to fermentation and recycling in fermentations, the yeast cells are exposed to different stresses simultaneously and consecutively. In this study, the brewing yeast was tested for tolerance to stressful fermentation conditions through alterations in the lipid composition of the mitochondria, which are often called cell power plants and are well developed and functional – for the laboratory strains – only in aerobic conditions. Since the neutral lipid composition of the mitochondria was unusual, we determined it in the plasma membrane as well. Non-stressed yeast culture, designated zero generation, was used as a reference.[28]

3.1 Phospholipids

Mass ratio of total PLs to proteins accounted for 0.020 mg mg^{-1} in the mitochondria of the starter culture and 0.027, 0.031 and 0.066 mg mg^{-1}, respectively, in the first three recycled generations.

Phospholipid composition of the mitochondria was characterised by predominance of PtdCho, which was the main PL in the zero and first two recycled generations (ranging from 23–29 %) and the second one in the third generation, accounting for 20 %. In spite of anaerobic conditions, CL was present in relatively high concentrations (14–16 %), which is a characteristic of the well-developed mitochondria. PtdSer was present in the lowest concentrations, ranging from 5–7 %, which is in accordance with the literature since it is enriched in the PM. When the results of analysed yeast generations were plotted for individual PLs, regularity in the alteration became obvious: in the first generation, obtained after the first beer fermentation, the alteration was the greatest, followed by the alteration in the opposite direction in the second and third generations (Fig. 1).



The results represent the mean value of double analysis of three independent experiments; the results for zero generation are from *Čanadi Jurešić et al*.[29]
Rezultati predstavljaju srednju vrijednost dviju analiza najmanje tri nezavisna pokusa; rezultati za nultu generaciju su iz *Čanadi Jurešić et al*.[29]

Fig. 1 – Phospholipid composition of the bottom-fermenting brewer's yeast mitochondria of the zero and three recycled generations
Slika 1 – Fosfolipidni sastav mitohondrija pivskog kvasca donjeg vrenja nulte generacije i tri reciklirane generacije

The resultant profile has arc-shape (PtdCho, PtdIns) or inverted arc-shape (PtdEtn, PtdOH, CL), respectively, and points to the specific pattern of the brewer's yeast response to unfavourable conditions and recycling. The same pattern was observed in a number of other parameters of the lipid composition.[21,23]

3.2 Neutral lipids

In the neutral lipid fraction of the mitochondria and plasma membrane, ergosterol and squalene were the main

components. The results of the analysis are presented in Table 1.

Table 1 – Content of ergosterol and squalene in the mitochondria and plasma membrane of the zero and first three recycled generations of brewer's yeast

Tablica 1 – Sadržaj ergosterola i skvalena u mitohondrijima i citoplazmatskoj membrani nulte i prve tri reciklirane generacije pivskog kvasca

Yeast generation Generacija kvasca		Zero Nulta	1st 1.	2nd 2.	3rd 3.
		m(lipids) : *m*(proteins) / μg mg^{-1} *m*(lipidi) : *m*(proteini) / μg mg^{-1}			
mitochondria mitohondriji	ergosterol ergosterol	16.2*	23.0	24.8	10.7
	squalene skvalen	4.7*	7.4	7.6	2.4
plasma membrane citoplazmatska membrana	ergosterol ergosterol	18.4	11.0	10.6	4.2
	squalene skvalen	10.4	19.4	12.8	9.5

The results represent the mean value of double analysis of at least three independent experiments; *in *Čanadi Jurešić et al.*[29]

Rezultati predstavljaju srednju vrijednost dviju analiza najmanje tri nezavisna pokusa; **Čanadi Jurešić et al.*[29]

Ergosterol was present in a higher concentration (mass ratio of lipids to proteins 11–25 μg mg^{-1}) compared to squalene (2–8 μg mg^{-1}), but regarding the normal distribution of the lipids in the cells, squalene as a hydrocarbon should not be present in the membranous systems at all. We have previously determined high concentrations of squalene in the mitochondria and plasma membranes in a recycled generation of another industrial strain of brewer's yeast,[20] which we have explained as a way the yeast cells cope with anaerobic conditions. Probably, squalene is incorporated in the mid-plane of the lipid bilayer, thus decreasing membrane permeability, as was hypothesised for bacteria.[30] Since the key step in sterol synthesis, the formation of squalene epoxide from squalene, occurs in aerobic conditions, the finding of squalene in the mitochondria of the zero generation (aerobically propagated) was quite unusual.[29] Squalene was found in the plasma membranes of all analysed generations, as well, and its concentration was higher than in the mitochondria accounting for 10–19 μg lipids/mg proteins (Table 1). In the plasma membrane of all recycled generations, its concentration was markedly higher than the concentration of ergosterol. These results confirmed our hypothesis that squalene replaced ergosterol in the membranes of the bottom-fermenting brewer's yeast in anaerobic and aerobic conditions, as well, as adaptation to the governing conditions during beer fermentation, which was achieved by careful strain selection during thousands of years.

3.3 Fatty acids

Several studies have reported on the fatty acid composition of brewer's yeast along with its alterations under different conditions, mainly in relation to the ethanol concentration.[18] The general conclusion for *S. cerevisiae* is that the composition of unsaturated FAs is a significant determinant of ethanol tolerance, and that oleic acid is the most efficacious unsaturated FA in overcoming the toxic effect of ethanol.[31] Published data concerning the mitochondria, are scarce. In our previous study of recycled yeast (the third generation) of another industrial strain of the bottom-fermenting brewer's yeast, we have determined palmitic acid as by far the most represented, accounting for 43 % (and 41 % in the plasma membrane, respectively). The overall content of saturated acids was very high, accounting for 79 % and the C16/C18 ratio was 5.5.[20] In the yeast analysed in this study, two acids were highly represented, palmitic and palmitoleic, accounting for 33–37 %, and 28–39 %, respectively (Table 2). Compared to the zero generation, the first recycled generation was characterised by a marked increase of palmitoleic acid mainly on the account of palmitic and oleic acid, which resulted in a marked increase in the C16/C18 ratio from 2.4 to 3.3. Similar to the plasma membranes, unsaturated fatty acids slightly predominated in all analysed generations, ranging from 53 % to 56 %, which was quite unusual for anaerobically grown

Table 2 – Main fatty acids in the total mitochondrial lipids of the zero and first three recycled generations of the bottom-fermenting brewer's yeast, and the main features of the composition (expressed as mass fraction of total identified fatty acids)

Tablica 2 – Glavne masne kiseline u ukupnim lipidima mitohondrija nulte i prve tri reciklirane generacije pivskog kvasca donjeg vrenja (izraženo kao maseni udjel ukupnih identificiranih masnih kiselina) te glavne značajke sastava

	Yeast generation Generacija kvasca			
	zero* nulta*	1st 1.	2nd 2.	3rd 3.
Fatty acid Masna kiselina	mass fraction / % maseni udjel / %			
C16:0	37.2	33.7	35.9	33.2
C16:1	31.6	38.6	27.7	36.8
C18:0	6.9	7.8	9.1	8.5
C18:1	18.9	10.2	12.1	10.5
C18:2	3.2	3.5	10.1	7.4
saturated, SFAs zasićene, SFAs	45.5	45.8	47.3	43.7
unsaturated, UFAs nezasićene, UFAs	54.5	54.2	52.7	56.3
UFAs/SFAs	1.2	1.2	1.1	1.3
unsaturation index indeks nezasićenosti	0.59	0.58	0.62	0.65
C16	68.7	72.3	63.7	70.0
C18	29.0	21.7	32.1	27.0
C16/C18	2.4	3.3	2.0	2.6
C16:0/C16:1	1.2	0.9	1.3	0.9
C18:0/C18:1	0.4	0.7	0.8	0.8

The results represent the mean value of double analysis of at least three independent experiments; *in *Čanadi Jurešić et al.*[29]

Rezultati predstavljaju srednju vrijednost dviju analiza najmanje tri nezavisna pokusa; **Čanadi Jurešić et al.*[29]

recycled generations.[23] In contrast to some other parameters of the lipid composition (e.g. phospholipid composition), the alterations of UFAs/SFAs were small and showed no trend, but the unsaturation index showed a marked increase in the second and third generations (Table 2). The FA analysis of individual PL classes showed that UFAs prevailed only in the main PLs, PtdCho and PtdEtn, of the first generation, while SFAs prevailed in the second generation and in other, less represented PLs (data not shown). These results suggest that neutral lipids were the pool of UFAs in recycled generations, which is in accordance with the findings of *Šajbidor and Grego*.[32]

4 Conclusion

Alterations in the lipid composition of the mitochondria under unfavourable conditions – which come to the fore mostly during transition from aerobic propagation to anaerobic beer fermentation – are marked in all lipid classes. On the other hand, recycling has a strong effect on phospholipids and neutral lipids, but a very slight effect on the FA composition. Therefore, since unsaturation is generally considered as the most important factor in the yeast adaptation to ethanol, we may say that the recycled yeast had adapted well to the governing conditions during the production process. The changes that follow transition from propagation to fermentation may be considered as stress injury, and alterations in the first recycled generations as stress response and indicator of good adaptation of the yeast to new, unfavourable conditions.

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List of abbreviations
Popis kratica

CL – cardiolipin
– kardiolipin

FA – fatty acid
– masna kiselina

FAME – fatty acid methyl ester
– metilni ester masne kiseline

PL – phospholipid
– fosfolipid

PtdOH – phosphatidic acid
– fosfatidna kiselina

PtdCho – phosphatidylcholine
– fosfatidilkolin

PtdEtn – phosphatidylethanolamine
– fosfatidiletanolamin

PtdIns – phosphatidylinositol
– fosfatidilinozitol

PM – plasma membrane
– citoplazmatska membrana

PtdSer – phosphatidylserine
– fosfatidilserin

SE – steryl ester
– sterolni ester

TAG – triacylglycerol
– triacilglicerol

TL – total lipids
– ukupni lipidi

TPL – total phospholipids
– ukupni fosfolipidi

UI – unsaturation index, %
– indeks nezasićenosti, %

m – mass, mg, μg
– masa, mg, μg

w – mass fraction, %
– maseni udjel, %

References
Literatura

1. *V. Marić, Z. Nadvornik*, Opis procesa proizvodnje piva, in D. Karlović (ed.), Pivo – tekuća hrana. Prehrambeno-tehnološki inženjering, Zagreb, 1995, pp. 19–30.
2. *B. R. Gibson, S. J. Lawrence, J. P. Leclaire, C. D. Powell, K. A. Smart*, Yeast responses to stresses associated with industrial brewery handling, FEMS Microbiol Rev. **31** (5) (2007) 535–569, doi: https://doi.org/10.1111/j.1574-6976.2007.00076.x.
3. *K. Sigler, D. Matoulkova*, Stress responses in brewing yeast, Kvasny Prum **57** (7-8) (2011) 277–284, doi: https://doi.org/10.18832/kp2011032.
4. *J. Bohunicki, B. Eržen, V. Marić, M. Pirkić, N.Todorović*, Kvasci i plijesni in Priručnik za mikrobiologe u pivarstvu, Poslovno udruženje industrije piva, Beograd, 1972.
5. *H. M. Walker-Caprioglio, W. M. Casey, L. W. Parks, Saccharomyces cerevisiae* membrane sterol modifications in response to growth in the presence of ethanol, Appl. Environ. Microbiol. **56** (9) (1990) 2853–2857.
6. *H. Alexandre, I. Rousseaux, C. Charpentier*, Ethanol adaptation mechanisms in *Saccharomyces cerevisiae,* Biotechnol. Appl. Biochem. **20** (1994) 173–183, doi: https://doi.org/10.1111/j.1470-8744.1994.tb00312.x.
7. *Z. Chi, N. Arneborg*, Relationship between lipid composition, frequency of ethanol-induced respiratory deficient mutants, and ethanol tolerance in *Saccharomyces cerevisiae*, J. Appl. Microbiol. **86** (1999) 1047–1052, doi: https://doi.org/10.1046/j.1365-2672.1999.00793.x.
8. *E. Bravi, P. Benedetti, O. Marconi, G. Perretti*, Determination of free fatty acids in beer wort, Food Chem.**151** (2014) 374–78, doi: https://doi.org/10.1016/j.foodchem.2013.11.063.
9. *G. M. Walker*, Yeast Growth, in G. M. Walker (Ed.), Yeast Physiology and Biotechnology, John Wiley & Sons, Baffins Lane, Chichester, 2000, pp. 101–202.
10. *H. A. Boumann, J. Gubbens, M. C. Koorengevel, C. S. Oh, C. E. Martin, A. J. R. Heck, J. Patton-Vogt, S. A. Henry, B. de Kruijff, A. I. P. M. de Kroon*, Depletion of Phosphatidylcholine in Yeast Induces Shortening and Increased Saturation of the Lipid Acyl Chains: Evidence for Regulation of Intrinsic Curvature in a Eukaryote, Mol. Biol. Cell **17** (2006) 1006–1017, doi: https://doi.org/10.1091/mbc.E05-04-0344.
11. *Z. Chi, S.D. Kohlwein, F. Paltauf*, Role of phosphatidylinositol (PI) in ethanol production and ethanol tolerance by a high ethanol producing yeast, J. Ind. Microbiol. Biotechnol. **22**

(1999) 58–63, doi: https://doi.org/10.1038/sj.jim.2900603.
12. *C. Osman, D.R. Voelker, T. Langer*, Making heads or tails of phospholipids in mitochondria, J. Cell Biol., **192** (1) (2011) 7–16, doi: https://doi.org/10.1083/jcb.201006159.
13. *S. E. Horvath, G. Daum*, Lipids of Mitochondria, Prog. Lipid Res. **52** (4) (2013) 590–614, doi: https://doi.org/10.1016/j.plipres.2013.07.002.
14. *M. Spanova, G. Daum*, Squalene – biochemistry, molecular biology, process biotechnology and applications, Eur. J. Lipid Sci. Technol. **113** (11) (2011) 1299–1320, doi: https://doi.org/10.1002/ejlt.201100203.
15. *B. Blagović, J. Rupčić, M. Mesarić, V. Marić*, Lipid analysis of the plasma membrane and mitochondria of brewer's yeast, Folia Microbiol. **50** (1) (2005) 24–30, doi: https://doi.org/10.1007/BF02931290.
16. *S. Rosenberger, G. Daum*, Lipids of yeast mitochondria, in G. Daum (Ed.), Cell Biology and Dynamics of Yeast Lipids. Research Signpost, Trivandrum, Kerala, India, 2005, pp. 51–83.
17. *T. M. Swan, K. Watson*, Stress tolerance in a yeast lipid mutant: membrane lipids influence tolerance to heat and ethanol independently of heat shock proteins and trehalose, Can. J. Microbiol. **45** (1999) 472–479, doi: https://doi.org/10.1139/w99-033.
18. *K. M. You, C.-L. Rosenfield, D. C. Knipple*, Ethanol tolerance in the yeast *Saccharomyces cerevisae* is dependent on cellular oleic acid content, Appl. Environ. Microbiol. **69** (3) (2003) 1499–1503, doi: https://doi.org/10.1128/AEM.69.3.1499-1503.2003.
19. *B. Blagović, J. Rupčić, M. Mesarić, K. Georgiu, V. Marić*, Lipid Composition of Brewer's Yeast, Food Technol. Biotech. **39** (3) (2001) 175–181.
20. *B. Blagović, J. Rupčić, M. Mesarić, V. Marić*, Lipid analysis of the plasma membrane and mitochondria of brewer's yeast, Folia Microbiol. **50** (1) (2005) 24–30, doi: https://doi.org/10.1007/BF02931290.
21. *G. Čanadi Jurešić, B. Blagović, J.* Rupčić, Alterations in Phosphatidylcholine and Phosphatidylethanolamine Content During Fermentative Metabolism in *Saccharomyces_cerevisiae* Brewer's Yeast, Food Technol. Biotechnol. **47** (3) (2009) 246–252.
22. *J. Rupčić, G. Čanadi Jurešić, B. Blagović*, Influence of stressful fermentation conditions on neutral lipids of a *Saccharomyces cerevisiae* brewing strain, World J. Microb. Biot. **26** (7) (2010) 1331–1336; erratum in **29** (10) (2013) 1975–1975, doi: https://doi.org/10.1007/s11274-009-0297-7.
23. *G. Čanadi Jurešić, B. Blagović*, The influence of fermentation conditions and recycling on the phospholipid and fatty acid composition of the brewer's yeast plasma membranes, Folia microbiol. **56** (3) (2011) 215–224, doi: https://doi.org/10.1007/s12223-011-0040-2.
24. *E. Zinser, G. Daum*, Isolation and biochemical characterization of organelles from the yeast, *Saccharomyces cerevisiae*, Yeast **11** (6) (1995) 493–536, doi: https://doi.org/10.1002/yea.320110602.
25. *H. Pichler, B. Gaigg, C. Hrastnik, G. Achleitner, S. D. Kohlwein, G. Zellnig, A. Perktold, G. Daum,* A subfraction of the yeast endoplasmic reticulum associates with the plasma membrane and has a high capacity to synthesize lipids, Eur. J. Biochem. **268** (8) (2001) 2351–2361, doi: https://doi.org/10.1046/j.1432-1327.2001.02116.x.
26. *O. H. Lowry, N. J. Rosenbrough, A. L. Farr, R. J. Randall*, Protein measurement with the Folin phenol reagent, J. Biol. Chem. **193** (1951) 265–275.
27. *J. Folch, M. Lees, G. H. Sloane Stanley*, A simple method for the isolation and purification of total lipids from animal tissues, J. Biol. Chem. **226** (1) (1957) 497–509.
28. G. *Daum, G. Tuller, T. Nemec, C. Hrastnik, G. Balliano, L.Cattel, P. Milla, F. Rocco, A. Conzelmann, C. Vionnet, D. E. Kelly, S. Kelly, E. Schweizer, H. J. Schüller, U. Hojad, E. Greiner, K. Finger*, Systematic analysis of yeast strains with possible defects in lipid metabolism, Yeast **15** (7) (1999) 601–14, doi: https://doi.org/10.1002/(SICI)1097-0061(199905)15:7<601::AID-YEA390>3.0.CO;2-N.
29. *G. Čanadi Jurešić, S. Šegota, M. Glad, B. Blagović*, Lipid composition of mitochondria of aerobically grown baker's and brewer's yeast, Curr. Trends Microbiol. **10** (2016) 105–113.
30. *T. Hauss, S. Dante, N. A. Dencher, T. H. Haines,* Squalene is in the midplane of the lipid bilayer: implications for its function as a proton permeability barrier, Biochim. Biophys. Acta-Bioen. **1556** (2-3) (2002) 149–154, doi: https://doi.org/10.1016/S0005-2728(02)00346-8.
31. *Z. Ciesarova, D. Šmogrovičova*, Štúdium etanoltolerancie kvasiniek, Chem. Listy **90** (1996) 365–370.
32. *J. Šajbidor, J. Grego*, Fatty acid alterations in *Saccharomyces cerevisiae* exposed to ethanol stress, FEMS Microbiol. Letters **93** (1992) 13–16, doi: https://doi.org/10.1111/j.1574-6968.1992.tb05033.x.

SAŽETAK

Utjecaj okolišnih čimbenika stresa i recikliranja na lipidni sastav mitohondrija pivskog kvasca

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Cilj istraživanja bio je odrediti promjene u sadržaju i sastavu lipida mitohondrija pivskog kvasca do kojih dolazi za vrijeme vrenja i recikliranja biomase. U radu je upotrijebljen pivski kvasac donjeg vrenja vrste *Saccharomyces cerevisiae* dobiven u industrijskom procesu. Analizirane su prve tri generacije recikliranog kvasca. Razlike između pojedinih generacija bile su izraženije u sastavu fosfolipida i neutralnih lipida nego u sastavu masnih kiselina. Skvalen je u svim generacijama bio prisutan u relativno visokoj koncentraciji. Rezultati istraživanja pružaju uvid u odgovor stanica kvasca na stresne faktore i recikliranje.

Ključne riječi
Pivski kvasac, recikliranje, mitohondriji, citoplazmatska membrana, lipidi, skvalen

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